Vascularized Composite Allotransplantation

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Upper Extremity Transplantation in Non-Human Primates: An Orthotopic Model for Translational Research

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Abbreviations: AO, The AO Foundation; ATGAM, antithymocyte globulin (equine); FK506, tacrolimus; IACUC, Institutional Animal Care and Use Committee; IM, intramuscular; IV, intravenous; LCDCP, Low Contact Dynamic Compression Plate; MHC, Major Histocompatibility Complex; MMF, Mycophenolate Mofetil; OR, operating room; PM, post mortem; POD, post-operative day; VCA, vascularized composite allotransplantation; VCs, venae commitantes.

Vascularized composite allotransplantation (VCA) offers unparalleled restoration of function and form following devastating musculoskeletal and soft tissue injury. However, the potential adverse effects of life-long immunosuppression remain a significant cause for concern. Therefore, while the surgical techniques necessary for VCA have developed rapidly, the immunological aspects of these procedures and the potential functional significance of immunological processes on vascularized composite allografts remain areas in which further research is required. The functional complexity of these procedures, combined with the preclinical nature of many of the research questions, necessitates the use of large animal models to most effectively address some of the outstanding hypotheses. Cynomolgus macaques are among the premier large animal models for immunological research. This manuscript describes development of an orthotopic model of upper extremity transplantation in cynomolgus macaques. Following study of the anatomy to determine feasibility, *in vivo* proof of concept was achieved by autologous amputation and replantation in two animals, following which a preliminary series of four allotransplants was performed. The anatomy encountered and techniques required for successful transplantation are closely comparable to those in clinical upper extremity transplantation. This is a technically challenging model, but offers a rigorous pre-clinical platform for translational research in transplant immunology, and is suitable for detailed study of the impact of immunologic processes on functional outcomes following VCA.

Introduction

Vascularized composite allotransplantation (VCA) is established as a treatment option in the management of complex musculoskeletal and soft tissue trauma and tissue loss. The utility of these procedures is particularly well illustrated by transplantation of specialized anatomical parts such as the upper extremity or those of the craniofacial region, where satisfactory replacement of like with like, consistent with Gillies' dictum,[1] using autologous tissue is extremely challenging and results often remain suboptimal.

Surgical development in VCA has been rapid, based on decades of experience in autologous free tissue transfer and microsurgery, and short to medium term results have been encouraging. Functional outcomes have been remarkable, particularly in the context of innervation, with hand transplant recipients appearing to benefit from a positive effect of calcineurin inhibition on peripheral nerve regeneration.[2] Recipients of both upper extremity and face transplants have reported significant improvements in independence and quality of life post-transplant. Graft survival rates following VCA have been largely encouraging, particularly for upper extremity transplants.[3] However this is offset by a

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high incidence of acute rejection episodes (up to 90% experience at least one episode within the first year) which most frequently target the skin,[3,4] and ongoing concerns regarding the side effect profile of conventional immunosuppression regimens. In this context, the necessity for further research into the immunologic responses to vascularized composite allografts, and development of novel clinical protocols with reduced risk, improved efficacy, or both is clear.

Murine models have been used extensively to gain important insights into the basic mechanisms of immune system function and the establishment and maintenance of immune tolerance.[5] More recently, advances in microsurgical techniques have facilitated the introduction of murine VCA models, allowing specific study of skin and musculoskeletal tissues in the context of vascularized composites.[6] However, historically, the vast majority of protocols which successfully achieve tolerance of a transplanted organ or tissue in small animal models fail to translate to large animals or to humans.[7] In contrast, mixed chimerism-based tolerance protocols originating from murine studies, and validated in non-human primate models,[8] have been successfully introduced to clinical trials in kidney transplantation.[9,10] Therefore, in addition to small animal studies, and porcine VCA models which we utilize extensively as a cost-effective large animal model uniquely well suited to the study of cutaneous immunobiology, we believe that rigorous non-human primate models are an important part of the translational research pathway in VCA.

Previous studies, performed prior to the introduction of clinical upper extremity transplantation, sought to investigate the technical feasibility of hand transplantation in baboons,[11] and of partial hand transplants (composites of the first ray and radial forearm flap) in rhesus macaques.[12] More recently, the radial forearm flap has been described as a model for study of VCA in cynomolgus macaques,[13] although this model lacked any functional component. Similarly, Barth and colleagues have described a model of heterotopic transplantation of partial facial allografts[14] which they have utilized in a number of studies of novel immunosuppressive regimens and the role of donor bone marrow in this context.[15,16]

This manuscript describes the methodology used for orthotopic upper extremity transplantation in cynomolgus macaques and presents results from our preliminary experience with this model, which permits detailed study of the immune status of recipients using the wide variety of validated immunologic techniques and reagents available for this species, including many cross-reactive human reagents. In addition the orthotopic transplantation of an upper extremity, with careful coaptation of nerves and tendons in a manner comparable to hand transplants in the clinical setting, offers the possibility of functional studies, including analysis of peripheral nerve testing and functional neuroimaging studies, which would not be possible in other model systems. However, it should be noted that this model presents a substantial technical challenge, requiring both surgical precision and careful postoperative management, which may be further complicated by the characteristics of the species utilized and which may limit its application in certain experimental settings.

Methods

All animal procedures described and demonstrated in this publication were conducted in accordance with protocols approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee (IACUC), in accordance with the Guide for the Care and Use of Laboratory Animals and under strict veterinary supervision.

1. Preoperative Planning and Preparation

1.1 Use cynomolgus macaques, purchased from approved vendors, with body weight greater than 6 kg. Animals should undergo thorough veterinary evaluation and quarantine prior to surgery. Select donor-recipient pairs on basis of size match, ABO blood group, and additional parameters as necessary for the experiment planned, including major histocompatibility complex (MHC) genotyping and expression of donor-specific markers for chimerism analysis.

1.2 In order to minimize ischemic time, procurement of the donor limb and recipient amputation should proceed in parallel. Plan surgery to ensure coordination of the surgical and anesthetic teams.

1.3 Confirm donor and recipient weights with anesthetic team 24 hours prior to transplant, and finalize orders for any non-anesthetic medications, such as induction immunosuppression, to be administered intraoperatively as required by the experimental protocol.

1.4 Check operating room equipment (critical equipment such as operating microscope and pneumatic tourniquet should be tested frequently and undergo routine preventative maintenance) and set up to facilitate work flow.

1.5 Ensure instruments are available and sterilized (autoclaved or gas sterilized) including reciprocating saw, drill, titanium plating set, microsurgical set, microvascular clamps and general dissecting instruments. In addition to donor and recipient operating tables with arm tables, back tables will be required for each animal, and for the sterile orthopedic equipment.

1.6 Withhold solid food for at least 12 hours prior to surgery for both donor and recipient. Water should be allowed ad libitum at all times.

2. Induction of Anesthesia and Intraoperative Monitoring

2.1 On the day of surgery administer premedication (Glycopyrolate 0.01 mg/kg) and sedative (Ketamine 20mg/kg) drugs IM.

2.2 Confirm adequate sedation by observation, remove animals from the cage and transfer to the OR. Remove hair from the surgical site (left arm) and additional sites for vascular access (contralateral arm, lower legs, tail) using clippers.

2.3 Place an appropriately size cuffed endotracheal tube and connect to anesthesia and ventilator circuit. Maintain anesthesia with isoflurane 0.5-3% and, for recipient animals, a continuous infusion of Ketamine 1mg/ml at a rate of 5-10 ml/hr.

2.4 Position animals on operating table with left arm extended on arm table, and pneumatic tourniquet in place. Place Bear Hugger forced air warmer to maintain body temperature under anesthesia. Lubricate eyes with veterinary eye ointment to prevent dryness under anesthesia.

2.5 Establish peripheral IV access in short saphenous veins and/or contralateral basilic veins. Administer maintenance fluids (0.9% normal saline, 10 ml/kg/hr) and medications intraoperatively.

2.6 Place a percutaneous arterial line in the ventral artery of the tail of recipient animals for continual invasive blood pressure monitoring.

2.7 Insert a self-retaining Foley urinary catheter (5 Fr) and connect to urometer to facilitate strict monitoring of fluid balance.

2.8 Establish monitoring of pulse, blood pressure, respiratory rate, pulse oximetry, capnography, EKG and temperature. In addition assess depth of anesthesia by assessment of jaw tone. Monitor and record these parameters throughout the period of anesthesia.

2.9 Administer preoperative analgesics (Buprenorphine 0.01 mg/kg IV, Banamine 1 mg/kg IM) and prophylactic antibiotics (Cefazolin 25 mg/kg IM).

2.10 Perform disinfectant skin preparation with solutions of chlorhexidine, povidone-iodine and alcohol and drape the surgical field.

3. Recipient Preparation

Induction immunosuppression may be administered intravenously to the recipient during the transplant procedure, however the details of immunosuppressive protocols are expected to be the focus of experimental studies using this model, and to therefore varying considerably. For this reason we have not included specific steps describing the administration of immunosuppressive reagents in this protocol.

3.1 Mark course of cephalic vein and other substantial cutaneous veins with surgical ink. Design and mark skin flaps to interdigitate with donor skin flaps approximately 4cm proximal to radiocarpal joint.

3.2 Elevate and compress arm to exsanguinate, inflate tourniquet to 200 mmHg.

3.3 Incise skin and elevate skin flaps, taking care to preserve cephalic and other cutaneous veins. Ensure hemostasis with bipolar diathermy.

3.4 Open investing fascia and dissect out radial artery and venae commitantes (VCs). Note that the radial artery may be bifid at this level in cynomolgus macaques with each branch accompanied by VCs.

3.5 Protecting cephalic vein and radial vascular bundle, divide the superficial digital flexors. Note that the musculotendinous junction extends further distally in comparison to human anatomy and division of muscle is likely at this level.

3.6 Identify, isolate and divide the median nerve. Section as far distal as possible and mark for later identification.

3.7 Isolate and protect the ulnar nerve and ulnar artery deep to flexor carpi ulnaris, which should be divided. Ligate the ulnar artery with clips and divide; this should be handled carefully as it may be required for secondary arterial anastomosis later. Divide ulnar nerve distally and mark for identification.

3.8 Divide the flexor digitorum profundi and the wrist flexors.

3.9 Divide the digital and wrist extensors.

3.10 Divide pronator quadratus and perform minimal periosteal stripping to prepare the osteotomy sites, which should be measured at 4cm from the radiocarpal joint.

3.11 Ligate and divide radial artery and cephalic vein. Amputate hand with reciprocating saw, taking care to perform well aligned, transverse osteotomies of radius and ulna.

3.12 Deflate tourniquet, ensure hemostasis with bipolar diathermy, ligature-clips and bone wax as necessary. Wrap stump in saline-soaked gauze and monitor recipient condition while awaiting donor hand.

4. Donor Operation – Allograft Procurement

Procurement of the donor hand proceeds in a similar manner to recipient amputation, with the exception that neurovascular structures sectioned distally in the recipient should be dissected proximally in the donor prior to division, to ensure sufficient length is available for tension-free anastomoses.

4.1 Mark course of cephalic vein and other substantial cutaneous veins with surgical ink. Design and mark skin flaps to interdigitate with donor skin flaps approximately 4cm proximal to radiocarpal joint (**Fig. 1 A**).

4.2 Elevate and compress arm to exsanguinate, and inflate tourniquet to 200 mmHg.

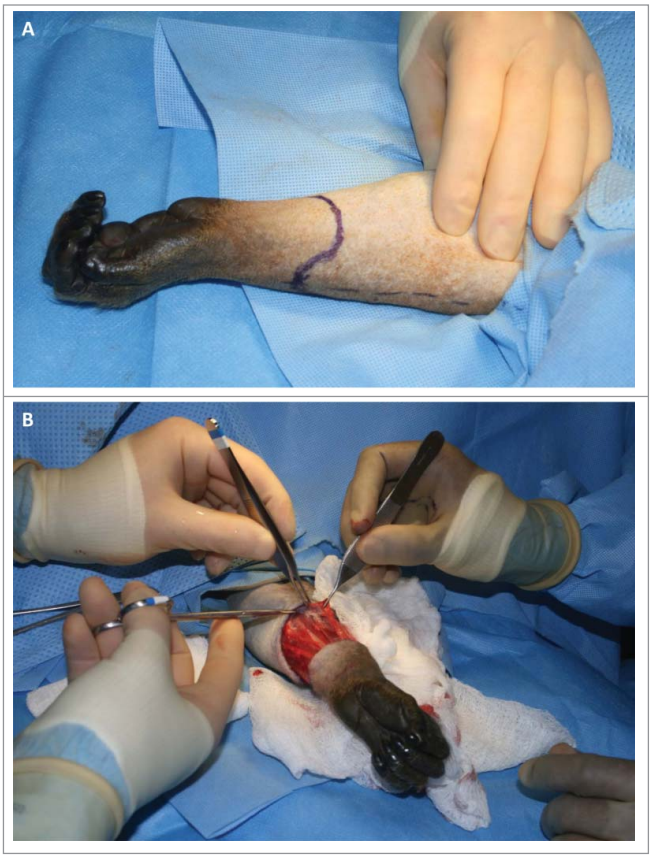

Figure 1. Marking and dissection for procurement of NHP donor hand. (**A**) Skin flaps are marked approximately 4cm from wrist on both recipient and donor to ensure skin flaps interdigitate properly following transplantation. Note that the course of the cephalic vein is also marked. (**B**) Dissecting proximally along the radial vascular bundle to procure additional length facilitates tension-free anastomosis.

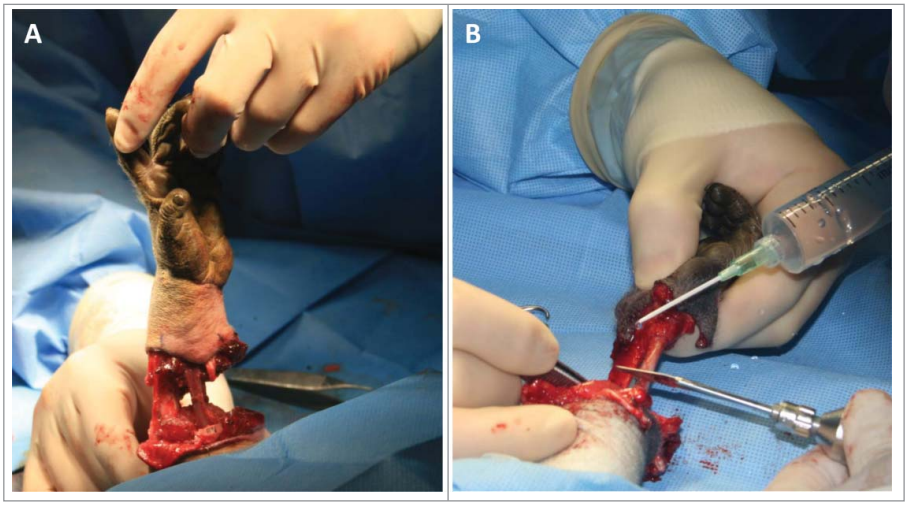

Figure 2. Isolation and amputation of the donor hand. (**A**) Following dissection and transection of all major structures except the radius, ulna, radial artery and cephalic vein the tourniquet is released, the donor heparinized and the hand reperfused for 20–30 minutes prior to procurement. (**B**) Following ligation and division of the radial artery and cephalic vein osteotomies are performed with a reciprocating saw.

4.3 Incise skin and elevate skin flaps, taking care to preserve cephalic and other cutaneous veins. Ensure hemostasis with bipolar diathermy.

4.4 Open investing fascia and dissect out radial artery and venae commitantes (**Fig. 1 B**).

4.5 Protecting cephalic vein and radial vascular bundle, divide the superficial digital flexors.

4.6 Identify, isolate and dissection out the median nerve, dividing it 1-2cm proximal to the level of the skin incision.

4.7 Divide flexor carpi ulnaris, taking care to protect ulnar nerve and artery lying along its deep margin. Divide ulnar artery and nerve proximally, and mark for identification.

4.8 Divide the flexor digitorum profundi and the wrist flexors.

4.9 Divide the digital and wrist extensors.

4.10 Divide pronator quadratus and perform minimal periosteal stripping to prepare the osteotomy sites, which should be measured at 4cm from the radiocarpal joint.

4.11 Deflate tourniquet, allowing hand to reperfuse via radial artery and cephalic vein (**Fig. 2 A**). Ensure hemostasis. Administer heparin 200 U/kg intravenously.

4.12 Allow hand to perfuse for 20-30 minutes, during which time a tray of sterile ice should be crushed and heparin-saline (100 U/ml) and perfusion solution prepared.

4.13 Ligate and divide radial artery and cephalic vein. The start of ischemic time should be noted.

4.14 Perform radial and ulnar osteotomies with reciprocating saw and remove hand to back table on ice (**Fig. 2 B**)

4.15 Humanely euthanize the donor animal and collect additional tissues as necessitated by your experimental plan.

Note 1: Two options are available for humane euthanasia; an intravenous overdose (200mg/kg) of sodium pentobarbital (Fatal Plus) administered under sedation, or exsanguination under deep anesthesia. Use the later only in experimental circumstances where vital bone marrow or other tissues to which sodium pentobarbital may be toxic must be collected.

Note 2: We routinely collect whole blood volume for isolation of leukocytes for *in vitro* assays and preparation of packed red cells for transfusion support of anemic animals, bone marrow for transplantation and induction of mixed chimerism, and split thickness skin grafts for later testing of tolerance.

5. Transplantation

5.1 With the hand on ice, cannulate the radial artery using an anterior chamber needle and 10ml syringe and flush with heparin-saline 100U/ml followed by perfusion solution (University of Wisconsin, or Euro-Collins) until venous effluent is clear.

5.2 Transfer hand to recipient table and ensure donor and recipient osteotomies are parallel and will permit well aligned rigid fixation.

5.3 Perform osteosynthesis with appropriately sized, titanium limited contact dynamic compression plates (LCDCP) and titanium screws. Use 2.0 mm plates for animals between 6 – 10kg. Fix ulna first with a 4-hole plate, followed by radius with a 6-hole plate, 8mm and 10mm screws respectively are appropriate in the majority of cases. Follow AO principles and ensure rigid fixation to minimize risk of non-union (**Fig. 3**).

5.4 Repair wrist flexors and extensors to stabilize wrist in a neutral position using 3/0 prolene or ethibond suture.

5.5 Repair deep digital flexor tendons to mimic natural digital cascade using 3/0 prolene or ethibond suture.

5.6 Bring in operating microscope and perform microvascular anastomoses of radial artery followed by cephalic vein. The artery can be expected to have a diameter of <1 mm and the vein 1-1.5 mm, 10/0 nylon suture is appropriate (**Fig. 4**).

Figure 3. NHP hand transplantation: Osteosynthesis. (**A**) Osteosynthesis of ulna and radius is performed using titanium LC-DCPs according to AO principles. Note microvascular clamps on proximal vessels in preparation for anastomosis. (**B**) AP and (**C**) Lateral radiographs demonstrating close approximation and good alignment of radius and ulna post-transplant.

5.7 Remove microvascular clamps and allow hand to reperfuse. Note end of ischemic time. Apply lidocaine 1% to vessels to relieve vasospasm, ensure recipient is well hydrated with adequate blood pressure and body temperature, and apply warm saline soaked gauze wraps to the hand. Allow hand to reperfuse for 20-30 minutes during which time the surgical team may rotate or take a short rest.

5.8 Assess perfusion. If adequate (hand warm, bright red bleeding from fingertip following needle prick) proceed with subsequent steps. If inadequate examine anastomoses under microscope for patency, ensure vessel lengths are appropriate to exclude both excess tension and kinking, and if necessary perform additional anastomoses to enhance perfusion. A secondary branch of the radial artery, or the ulnar artery are available, additional dorsal veins may be identified. The venae commitantes of the arteries are of insufficient caliber for reliable anastomosis.

5.9 Perform median and ulnar neurrorhaphies, aiming to do so as far distal as possible, using 8/0 or 9/0 nylon suture.

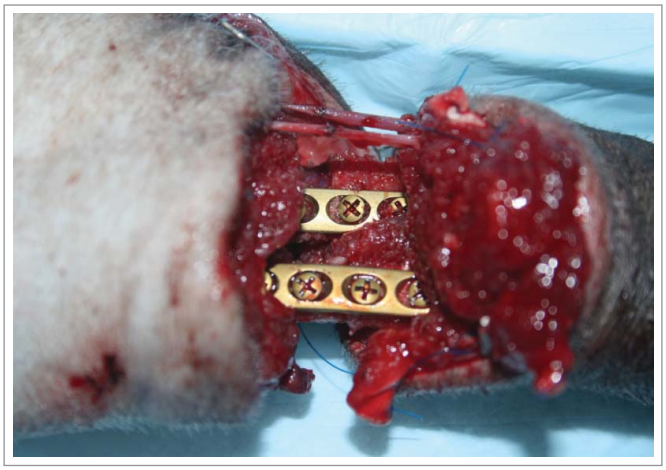

Figure 4. NHP hand transplantation: Reperfusion. Following anastomoses of radial artery and cephalic vein, visible above plates in this photograph, the hand is reperfused for 20–30 minutes and perfusion assessed prior to continuing with repair of flexor and extensor tendons, median and ulnar nerves and closure of skin flaps.

Figure 5. Post-transplant appearance: (**A**) Radial-oblique and (**B**) ulnar-oblique views immediately following closure of skin flaps. (**C**) Bright red bleeding from fingertip following needle-prick confirms the hand is well perfused prior to application of dressings and protective cast.

5.10 Repair superficial digital flexor tendons with 3/0 prolene or ethibond using low-profile side-to-side technique.[17]

5.11 Perform extensor tenorrhaphies using 3/0 prolene or ethibond, setting tension to allow full range of motion and maintaining digital cascade.

5.12 Interdigitate and close skin flaps using 3/0 nylon simple interrupted sutures, taking care to avoid damage to the cephalic vein or arterial anastomoses (**Fig. 5 A, B**)

5.13 Check perfusion adequate (**Fig. 5 C**) before applying dressings and cast and recovering the animal to its cage.

Note that this protocol may be adapted to perform amputation and autologous replantation if required to address specific experimental aims, by following the procedures for donor procurement and transplantation on a single animal. Shortening osteotomies of 5-10mm to radius and ulna may be necessary to relieve tension on neurovascular structures and facilitate anastomoses.

6. Postoperative Care

6.1 Dress hand and wound with gauze and bulky wool, and apply a full-length fiberglass cast to the arm with the wrist slightly extended, and the metacarpophalangeal and interphalangeal joints loosely flexed in a natural cascade position.

Note: The elbow should be slightly flexed to prevent effortless removal of the cast, while simultaneously avoiding vascular constriction at the antecubital fossa.

6.2 Place animal in a cotton jacket. Secure cast to front of jacket to achieve relief from dependent position.

Note: Animals must be acclimated to wearing of jackets and casts prior to commencing study.

6.3 Ensure adequate hydration and analgesia. If placed in protective jacket, a fentanyl patch 1–4 μg/kg/hr may be placed to provide postoperative analgesia. Alternatively buprenorphine 0.01 mg/kg IM must be administered twice daily for 72 hours, more frequently if clinical signs of pain are observed.

6.4 Wean from anesthesia, discontinue monitoring.

6.5 Return to cage, which should be warmed with a heat lamp, and monitor continually until fully alert and recovered from anesthesia.

6.6 Perform examination of the transplanted hand daily or twice daily for the first 72 hours postoperatively to ensure adequate perfusion and facilitate rapid intervention if necessary to revise anastomoses etc. Short duration sedation for these procedures may be induced with intramuscular injection of ketamine (1-4 mg/kg) and dexmedetomidine (5-10 μg/kg), followed by atipamezole reversal. The frequency of checks can be reduced to twice weekly after the immediate postoperative period.

Table 1. Experimental design and animal data

Group	Animal ID Number	Sex	Weight (kg)	Recipient Blood Group	Donor Blood Group	MHC Mismatch
I	PM Tissue #1	M	9.0	N/A	N/A	N/A
	PM Tissue #2	M	8.5	N/A	N/A	N/A
II	M5612	M	6.8	AB	N/A	Auto (replant)
	M5712	M	8.2	AB	N/A	Auto (replant)
III	M1413	M	6.5	A	A	Full
	M5812	M	7.5	AB	AB	MHCI single haplotype shared
	M4313	M	8.5	B	B	Full
	M4213	M	9.2	B	B	Full

6.7 Perform 2-view radiographs on post-operative day 1 to ensure fixation is appropriate and the position acceptable.

6.8 Administer immunosuppressive medications according to individual experimental protocols., Most conventional immunosuppressive agents may be administered daily via a combination of IM and oral routes. Draw blood twice weekly to monitor drug levels. In this study, in the allotransplant group, immunosuppression was induced with 3 consecutive daily doses of equine anti-thymocyte globulin (ATGAM) 50 mg/kg IV, and maintained with FK506 0.1 mg/kg/day IM (target trough 15-20 ng/ml), MMF 300 mg/day and methylprednisolone tapered over the first 2 weeks from 40 mg/day to a maintenance dose of 1 mg/day.

Experimental Design

The aim of this study was to develop a model of orthotopic upper extremity transplantation in cynomolgus macaques. The first step in this process was to perform post-mortem dissections to confirm the anatomy of the cynomolgus monkey forearm and to determine the feasibility of transplantation and revascularization (2 animals, group I). We then performed autologous replantation in 2 animals for *in vivo* proof of technical feasibility (group II). Finally, a preliminary study of 4 allotransplants across full MHC barriers was performed (group III). The experimental design and details of the animals in each group are summarized in **Table 1.**

Representative Results

Post-mortem dissections (in animals euthanized at the conclusion of their experimental course on other protocols) confirmed the anatomy of the cynomolgus monkey forearm was closely conserved with that of humans. A few exceptions were noted, chiefly that in both animals examined, the arterial supply to the hand was provided by 2 arteries in the radial compartment of the wrist, the medial of which arose from the radial artery in the distal third of the forearm. The ulnar system appeared to be vestigial, appearing as a vasonervorum to the ulnar nerve. The radius had a mean diameter of 10 mm and the ulna 5mm, suitable for osteosynthesis with 2 mm plates and screws.

Satisfied with the feasibility of transplantation and revascularization, we proceeded to perform a series of 2 autologous replants and 4 allotransplants across full MHC barriers. Total operating time averaged 11–12 hours. Mean ischemic time in both *in vivo* groups was 3 hours. All microvascular anastomoses were performed using conventional suture techniques for both artery and vein, which had diameters of 0.75–1 mm and 1–1.5 mm respectively. All anastomoses were demonstrably patent with no primary failures.

In the autologous replantation group (group II), one animal unfortunately developed respiratory complications under anesthesia, secondary to endotracheal tube trauma, from which resuscitation could not be achieved. The second animal in this group tolerated the procedure well, made impressive functional recovery, including use of the hand in locomotion, grooming and as an assist hand while feeding and manipulating objects. This animal remains under follow up over 2 y post-operatively.

Outcomes following allotransplantation (group III) have highlighted the critical importance of precise microsurgical technique, and the challenges of microvascular procedures on this small scale in animal models. Two animals unfortunately required early euthanasia due to microvascular compromise; in one case excess length following venous anastomosis permitted the development of kinking and venous thrombosis on POD2. Despite emergency revision, a no-reflow state had developed and the hand could not be reperfused. In another case, despite patent anastomoses, and treatment with topical lidocaine 1% for relief of vasospasm, perfusion of the hand was poor postoperatively and despite medical optimization, warming, and anticoagulation perfusion remained poor. Persisting ischemia declared over the first postoperative week and the animal was euthanized on POD7.

The remaining 2 animals in the initial allotransplant group had smooth operative courses, and made excellent post-operative recoveries. The first was followed to an experimental end point 4 months post-transplant, during which period immunosuppression was maintained with FK506 0.1 mg/kg/day, MMF 300 mg/day and methylprednisolone tapered from over the first 2 weeks from 40 mg/day to a maintenance dose of 1 mg/day, following ATGAM induction. This regimen

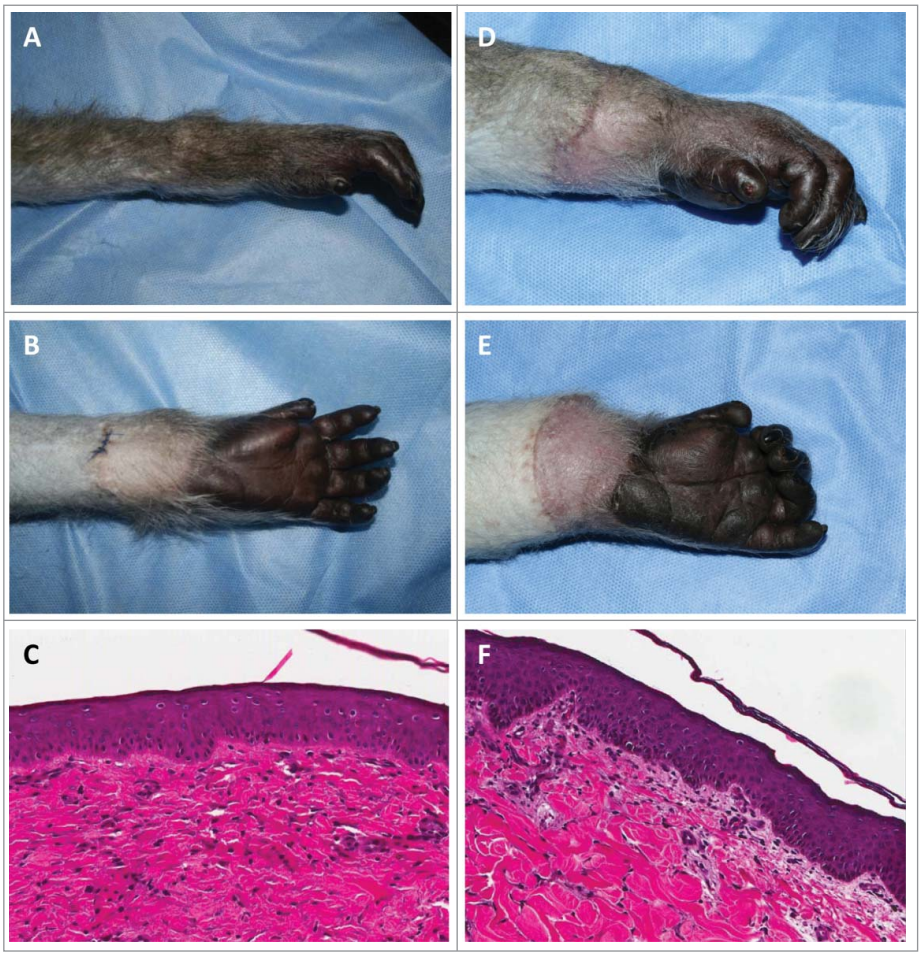

Figure 6. Representative result: (**A**) Radial and (**B**) Palmar views demonstrating status 3 months post hand transplantation (M1413). Note sutures following protocol biopsy, which confirmed rejection-free status. (**C**) Hand transplant biopsy specimen demonstrating absence of rejection. Immunosuppression was maintained with FK506 0.1 mg/kg/day, MMF 300 mg/day and Methylprednisolone 1 mg/day. (**D**) Radial and (**E**) Palmar views demonstrating appearance of hand during acute rejection (M4213, POD30) (**F**) Corresponding histology demonstrating Banff grade I acute cellular rejection.

resulted in rejection-free survival (**Fig. 6A–C**), with the exception of 1 episode of acute rejection of skin which followed tapering of MMF to 100 mg/day due to a period of inappetence. Signs of rejection resolved rapidly in response to steroid bolus and return to standard MMF dosing. The final animal in this group experienced a smooth interoperative course, and the hand reperfused rapidly on completion of vascular anastomoses. A surgical site infection was diagnosed during the second post-operative week following observation of a suture line abscess and local erythema of adjacent transplanted and recipient skin. This resolved promptly with antibiotic therapy, and the hand remained well perfused with no signs of inflammation until POD 30, when mild generalized erythema of the donor skin was noted (**Fig. 6D, E**). A biopsy was taken and rescue therapy commenced with a corticosteroid pulse. The biopsy confirmed Banff I acute rejection (**Fig. 6E**). Despite continued high-dose corticosteroid treatment, rejection was progressive resulting in graft loss and humane euthanasia on POD51.

Discussion

Non-human primate models are widely considered the final step on the translational research pathway prior to clinical trial. Positive results in such models are viewed with particular importance in fields, such as VCA, where results of current therapies are acceptable, albeit not always optimal, and where patient survival is expected to be high without intervention – thus establishing that the ethical standard for any new therapy should be beneficence rather than non-maleficence.

Previously described non-human primate models of VCA have achieved transplantation of the necessary tissue types, and have provided insights into the immune response to these tissues under conventional immunosuppression,[13,14] and some novel protocols.[15] However, these models include no functional component by which to assess this important aspect of VCA. This orthotopic upper extremity transplantation model, in contrast, is closely analogous to clinical hand transplant procedures, and provides a unique opportunity to study not only the immune response to the transplanted tissues, but to investigate in detail the impact of rejection, or aspects of novel tolerance or immunomodulatory protocols on functional outcome. Representative results from such studies are not yet available, but investigation of peripheral nerve recovery, functional testing, and advanced imaging studies are planned.

This model is technically challenging, and presents a steep learning curve as illustrated by the results presented. High quality, rigid boney fixation is critical in avoiding non-union. Precise microvascular technique is also imperative, as we have found this model highly sensitive to even minor excess vessel length, particularly following venous anastomosis. Typically we observe satisfactory perfusion of the transplanted hand following anastomosis of radial artery and cephalic vein alone. In two cases we have performed secondary arterial anastomoses using branches of the radial artery, which interestingly, appears bifid in these animals. Venous drainage via the cephalic vein has generally been sufficient, with the notable exception of the case in which kinking and occlusion of the vein resulted in thrombosis, congestion and no-reflow phenomenon, however anastomosis

of one or more radial artery venae comitantes may provide a greater margin of safety in animals which do not tolerate elevation of the arm during the immediate postoperative period.

With regard to immunosuppression, in this preliminary study we have utilized a protocol of ATGAM induction followed by maintenance with tacrolimus, mycophenolate mofetil and methylprednisolone, adopted from experimental solid organ transplant protocols in macaques at our institution. This appears sufficient to prevent graft loss to rejection post-transplant, albeit requiring doses higher than typical in clinical use. The observation of progressive, steroid-refractory rejection in one animal is consistent with similar findings in solid organ transplantation models (Madsen J, unpublished data). Taken together these observations demonstrate that the cynomolgus macaque represents a robust preclinical model for transplantation research. Further studies are underway utilizing this model for investigation of clinically applicable tolerance strategies.

This model is resource intensive, requiring an appropriate non-human primate research facility, expert veterinary anesthetic support and a team of experienced reconstructive microsurgeons. Clearly structured rehabilitation to maximize functional outcomes is not possible in NHPs as it is in patients, and this could represent a limitation of this model when it comes to future functional studies. However we have been impressed by the considerable degree of function which animals recover spontaneously, including use of the hand in grooming, as an assist hand in feeding, and for locomotion. We believe this model represents a valuable tool in the effort to validate novel protocols to improve the immunologic management of VCA, and we hope, ultimately to facilitate the introduction of a safe and effective protocol for induction of VCA tolerance to clinical trial. However, the inherent technical and logistical challenges, not to mention the ethical obligations associated with research in nonhuman primates may limit application of this model to late-stage proof of concept studies immediately preceding translation to clinical trial.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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